STRATEGIC DIAGNOSTICS INC.

EnviroGard® Cyclodiene Test Kit

73300

Intended Use

The EnviroGard Cyclodiene Test Kit is a qualitative "yes/no" or semi-quantitative test for the detection of cyclodiene residues in water or other aqueous solutions. You can use the test kit in the laboratory or on-site without any special training. With the EnviroGard Cyclodiene Test Kit, you can read results visually or perform a more precise analysis with a photometer.

Test Principles

The EnviroGard Cyclodiene Test Kit is based on the use of polyclonal antibodies that bind either cyclodiene or chlordane-enzyme conjugate. Cyclodiene in the sample competes with chlordane-enzyme conjugate for a limited number of antibody binding sites. Antibodies that bind cyclodiene are immobilized to the walls of the test tubes.

Since there are the same number of antibody binding sites on every test tube and each test tube receives the same number of enzyme conjugate molecules, a sample that contains a low concentration of cyclodiene enables the antibody to bind many chlordane-enzyme conjugate molecules. Therefore, a low concentration of cyclodiene produces a dark blue sample. Conversely, a high concentration of cyclodiene enables the antibodies to bind fewer chlordane-enzyme conjugate molecules, resulting in a lighter blue sample.

NOTE: Color is inversely proportional to cyclodiene concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Performance Characteristics

The EnviroGard Cyclodiene Test Kit does not differentiate between the various cyclodiene compounds, but detects their presence to differing degrees. The following chart shows the approximate lower limit of detection, or Lower Limit of Detection (LLD), for the cross-reacting compounds. Concentration is in parts per billion (ppb).

Compound	LLD
Chlordane	2.0
Aldrin	3.0
Endosulfan	0.5
Endrin	0.5
Dieldrin	0.9
Heptachlor	3.0
Toxaphene	100.0

Precautions

- Store all test components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not store test components for more than 8 hours at ambient temperatures (18°C to 27°C or 64°F to 81°F).
- Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test (approximately 30-60 minutes).
- Do not use test kit components after the expiration date.
- Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.
- Because of the rapid kinetics of the EnviroGard Cyclodiene Test Kit, do not analyze more than six test tubes at one time.
- Use approved methodologies to confirm any positive results.
- Do not dilute or alter test reagents or samples not called for in the test procedure; this may give inaccurate results.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this test kit.
- Use a calibrator that has a matrix comparable to your sample if you are testing something other than water.

Materials Provided

The EnviroGard Cyclodiene Test Kit contains the following items:

20 Antibody-Coated Test Tubes
1 vial of Negative Control
1 vial of Chlordane-Enzyme Conjugate
1 vial of 10 microgram/milliliter (µg/mL) Chlordane Stock Solution
1 vial of Substrate
1 vial of Chromogen
1 vial of Stop Solution
1 Test Tube Rack

Materials Needed - Not Provided

You will also need several other items:

- marking pen for labeling test tubes
- disposable-tip pipette that will measure 100 microliters (µl) and 160 µl
- digital timer
- tap or distilled water for washing test tubes
- lab-grade water for calibrator preparation
- pipettes that will measure 0.5 ml, 0.9 ml, 0.99 ml, and 1.0 ml
- positive displacement pipette which will measure 10 µl
- volumetric flasks, 10 ml and 100 ml, or glass test tubes for calibrator preparation
- calculator (optional)
- SDI differential photometer (optional)

Calibrator Preparation

The kit contains a 1.0 mL vial of 10 µg/mL chlordane in methanol. ***Do not use this stock solution directly in the assay.*** Chlordane will adhere to glass surfaces; therefore, calibrators should be used within a few hours after preparation. Choose either of the following dilution schemes.

If all twenty test tubes are to be used on the day of the test:

1. Remove the cap on the 10 µg/mL Chlordane Stock Solution vial and carefully transfer the contents to a clean 100 mL volumetric flask. Carefully rinse the vial with a few milliliters of lab-grade water and add this to the flask. Fill the flask to 100 mL with lab-grade water. Label the flask "100 ppb Chlordane".

2. Transfer 1.0 mL of the 100 ppb solution to a clean glass 10 mL volumetric flask. Fill the flask to 10 mL with lab-grade water, cap, and mix thoroughly. Label the flask "10 ppb Chlordane".

If less than twenty test tubes are being used on the day of the test:

1. Using a 10 µL positive displacement pipette, remove 10 µL of the 10 µg/mL Chlordane Stock Solution and add to a clean glass test tube, which contains .99 mL of lab-grade water. Vortex to mix thoroughly. Label this tube "100 ppb Chlordane."
2. Add 100 µL of the 100 ppb solution to a clean glass test tube which contains 0.9 mL of lab-grade water. Vortex to mix thoroughly. Label this tube "10 ppb Chlordane."

Perform the Test

1. Remove the test tubes from the plastic bag and mark them as follows:

Tube Marking	Tube Contents
"–"	Negative Control
"+"	10 ppb or 100 ppb Calibrator
"S1"	Sample 1
"S2"	Sample 2
"S3"	Sample 3

2. Add 160 µL of Negative Control to the "–" test tube.
3. Add 160 µL of the appropriate calibrator to the "+" test tube.

NOTE: You can use both calibrators to approximate the concentration levels of your samples to a more accurate degree. However, you should not use more than six test tubes in one test. If you use both calibrators, label them accordingly ("10 ppb" and "100 ppb").

4. Add 160 µL of each sample to the corresponding test tube. Immediately add 4 drops (160 µL) of Chlordane-Enzyme Conjugate to each test tube. Gently swirl the test tubes to mix for 2 to 3 seconds.
5. Leave the test tubes undisturbed for 5 minutes, then shake out their contents.
6. Fill the test tubes to overflowing with tap or distilled water, then decant and vigorously shake out the remaining water.

NOTE: Repeat this wash step three more times, being certain to shake out as much water as possible after each wash.

7. Add 4 drops of Substrate to each test tube. Immediately add 4 drops of Chromogen to each test tube. Gently mix the test tubes for a few seconds.

CAUTION: Do not reverse this order; add the Substrate before the Chromogen.

Interpret the Results

You can either interpret the results visually within 3 minutes after adding the Substrate and Chromogen to each test tube or you can perform a more precise analysis with a photometer.

NOTE: If a blue color does not develop in the negative control test tube within 3 minutes after adding the Substrate and Chromogen, the test is invalid and you must repeat it.

Visual Interpretation

1. Compare the sample test tube to the calibrator tube(s) against a white background. The test tube rack in the kit is well-suited for this purpose.
2. If the sample test tube contains less color than a calibrator tube, the original sample contains cyclodiene at a concentration greater than or equal to the concentration of that calibrator.
3. If a sample test tube contains more color than a calibrator tube, the original sample contains a lower concentration than that calibrator.

Photometric Interpretation

After 3 minutes, add 1 drop of Stop Solution to each test tube and mix well. This turns the solution yellow.

WARNING: Stop Solution is 2.5 N sulfuric acid.

Conventional Spectrophotometers

1. Add 0.5 mL of water to each test tube and gently mix.
2. Adjust the wavelength of your photometer to 450 nanometers (nm) and zero against a water blank.
3. Transfer the reaction liquid to an appropriate cuvette or aspirate directly into the photometer (depending on your photometer type).
4. Measure and record the absorbance (optical density [OD]) of the negative control, each sample, and the calibrator.
5. Compare the OD of each sample to the OD of the calibrator. If the OD of the sample is less than the OD of the calibrator, the sample contains a concentration greater than the concentration of the calibrator. Conversely, if the OD of the sample is greater than the OD of the calibrator, the sample contains a concentration that is lower than the concentration found in that calibrator.

Differential Photometer

1. Add 0.5 mL of water to each test tube and gently mix.
2. Place a test tube containing water in the left (reference) well.
3. Place the negative control test tube in the right (sample) well. Record the OD of the sample.
4. Remove the negative control test tube and replace it with the next tube (calibrator or sample) to reactivate the photometer. Record the result. Repeat this procedure to determine the OD for each of the remaining samples.
5. Compare the OD of each sample to the OD of the calibrator. If the OD of the sample is less than the OD of the calibrator, the sample contains a concentration greater than the concentration of the calibrator. Conversely, if the OD of the sample is greater than the OD of the calibrator, the sample contains a concentration lower than the concentration of that calibrator.

Ordering Information

Description	Catalog Number

EnviroGard Cyclodiene Test Kit	73300

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General Limited Warranty
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